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Ex vivo evaluation of the mucoadhesive properties of Cedrela odorata and Khaya senegalensis gums with possible applications for veterinary vaccine delivery

B. Emikpe, V.O. Oyebanji*, T. Jarikre, D. Odeniyi, A. Salami, O. Oladele, O. Akinboade

University of Ibadan,, Ibadan, Nigeria

Background: The use of mucoadhesives in drug vehicle design has gained considerable attention over the past decades however, studies designed to evaluate the interaction of bioadhesives with biologic tissues with a view of application for non-invasive vaccine delivery in veterinary subjects' is scanty in literature. Hence, this study evaluates the peak adhesion time as well as capacity for vaccine delivery through the mucosal route of some phytogenic mucoadhesive polymers.

Methods & Materials: Gum gels from *Cedrela odorata* and *Khaya senegalensis* were harvested, purified, lyophilized and compressed into 500mg tablets individually and in combined ratios of 1:1, 1:3 respectively. These tablets were placed on freshly excised (about 5x5cm) trachea and duodenal tissues of cattle, chicken, pig, sheep and goat which were fastened to the basket end of a tablet dissolution machine probe. The probe set at 50rev/min was lowered into phosphate buffer at 6.8 pH in a beaker immersed in water bath at 37^{O}C. The time it takes for the gum tablet to fall off the tissue under this condition is recorded as the peak adhesion time (PAT) of the gum polymer. The gum polymer with the best PAT was combined with Newcastle disease vaccine and the procedure repeated. Haemagglutination assay (HA) was conducted on the gum polymer-vaccine mix with gum and vaccine individually as controls.

Results: On cattle, chicken and sheep tissues, Cedrela-Khaya (1:1) mix had the highest PAT; goat, Cedrela-Khaya (1:3) mix while either Cedrela-Khaya (1:1) mix or Cedrela alone was best for pig tissues. On combination with vaccine, the PAT of the gums reduced slightly on cattle and sheep tissues while other animal tissue showed varied results. The HA results showed the gum polymer boosted the HA property of the vaccine ($Log10^{5}$), when compared to vaccine alone ($Log\ 10^{4}$). There was presence of HA property in the gum polymer alone. With a checkerboard dilution, the minimum dilution with the least HA property was recommended for vaccine dilution and *in vivo* application.

Conclusion: In conclusion, mucoadhesives from phytogenic sources has potentials for non-invasive vaccine application with possible amplification of duration of immune vaccine response.

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Identification and characterization of a novel protein PfCDPK-5 for the development of pediatric malaria vaccine

D.K. Raj[1,*], S. Pond-Tor[1,2], B. Sherman[3], J. Kurtis[2]

[1] *Brown University, Providence, Rhode Island, USA*
[2] *Centre for International Health Research, Providence, USA*
[3] *Brown University, Providence, USA*

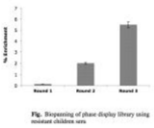
Background: Malaria are among the leading causes of mortality for children under five years of age worldwide, with most of these deaths resulting from *Plasmodium falciparum* infection. Resistance to existing anti-malarial medications is an urgent problem and may prevent effective eradication strategies. Despite decades of research, no vaccine candidate has been shown to confer significant protection to children. Though marginal protection has been achieved using the vaccine candidate RTS, S/AS01 and irradiated sporozoites, broadly effective vaccine candidates are urgently needed. The immediate goals of this study are to gain an immunological understanding of anti-PfCDPK-5 antibodies in preventing parasite maturation and egress.

Methods & Materials: In ongoing antigen discovery studies, we pioneered a high-throughput differential whole proteome screening method (phase display) to identify parasite epitopes associated with resistance to malaria in children. We utilized this approach to identify targets of antibodies that protect children from severe malaria or malaria-specific mortality and identified Schizont Egress Antigen-1 (Raj et. al Science 2014). In a parallel screening experiment, we used sera from 11 resistant and 14 susceptible children to differentially screen a parasite phage display cDNA library generated from parasite collected from African children. The localization study and growth inhibition activity were evaluated as per our published methods. Anti-rPfCDPK-5 antibody levels in the entire cohort measured by a bead-based assay.

Descriptive characteristics of Resistant and Susceptible children

	Variables	Resistance With Low PD	Susceptible	P value
1	Number of subjects	12	14	1
2	Haemoglobin Phenotype (% AS)	25	14.3	0.910
3	Sex (% female)	33	50	0.190
4	Length of follow -up (in weeks)	14.0[13.5]	17.0[14]	0.047
5	# Of blood smears from age 2-3.5 years	14[7.4]	2.2[8.5]	0.0102
6	# Of positive blood smears from age 2-3.5 years	2.1[1]	5.0[3.5]	0.0024
7	# Of anti-malarial treatment before 2	1.3[1.1]	2.3[3.1]	0.133
8	Pregnancy malaria (%)	25	21.4	0.300
9	Maternal age	27[9]	26.5[6]	0.833
10	Birth Season (% in high season)	41.7	42.9	0.952
11	Subjects using bed net (%)	0	100	<0.001
12	# Of previous pregnancies	2[2]	2.3[2.5]	0.760
13	# of peripheral parasites per 200 WBCs) at enrollment	0	0	1
14	Mean Parasite density (parasites per 200 WBCs) from age 0-2 years	623[952]	1400[736]	0.1569
15	Mean Parasite density (parasites per 200 WBCs) from age 2 -3.5 years	7.1[2.9]	2258[1569]	<0.001

Descriptive characteristics of Resistant and Susceptible children



Enrichment of antigens binding to malaria resistant kids antibodies

Results: We identified antibodies against PfCDPK-5 protein only in malaria resistant kids sera, not in susceptible kids sera. The preliminary data show that PfCDPK-5 phosphorylates PfSEA-1 another important protein responsible for egress of parasites. The poly-

clonal antibodies generated by recombinant PfCDPK-5 protein or DNA vaccine shows significant growth inhibition activity in *in vitro* assays. Anti-rPfCDPK-5 antibody levels in the entire cohort shows a positive correlation to morbidity and mortality of children.

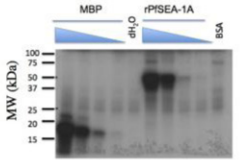


Fig . PfSEA-1A is phosphorylated by rPfCDPK-5 in *in vitro* kinase assay. Positive control substrate (MBP-myelin basic protein), rPfSEA-1A, and negative control substrate (BSA) were incubated with 0.25ug of rPfCDPK5, 1mM $CaCl_2$ and ^{32}P-ATP for 30 min at 37C followed by SDS-PAGE and auto-radiography. Lane 1, 1.0ug of MBP, lane 2, 0.1ug MBP, lane 3, 0.01ug MBP, lane 4, 0.001ug MBP, lane 5, no substrate, lane 6, 1.0ug rPfSEA-1A, lane 7, 0.1ug rPfSEA-1A, lane 8, 0.01ug rPfSEA-1A, lane 9, 0.001ug rPfSEA-1A, lane 10, 1.0ug of BSA. Band at 75 kDa in lanes 6 and 10 represents auto-phosphorylated rPfCDPK5.

PfCDPK-5 phosphorylate PfSEA-1

Conclusion: In the present study, we validate a rationally identified vaccine candidate, *P. falciparum* calcium-dependent protein kinase 5(PfCDPK-5) using integrated translational approaches that harness high-throughput molecular techniques and *in vitro* functional assays.

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Lesson learned from investigating cluster adverse event following immunization in mass campaign of Japanese Encephalitis vaccine in India

A.K. Singh[1,*], J. Joshi[2], A. Shewale[3], N. Gupta[4]

[1] *Public Health Foundation of India, Delhi, Delhi, India*
[2] *Public Health Foundation of India, Delhi, India*
[3] *MoHFW, New Delhi, India*
[4] *Public Health Foundation of India, New Delhi, India*

Background: Vaccine safety is one of the critical parameters for quality assurance in immunization program in all countries including developing country like India as it adds new vaccines to its existing immunization program. Occurrence of adverse events following immunization (AEFI) and spread of unchecked rumors can hamper community confidence in vaccines adversely affecting coverage. Cluster AEFI (2 or more reports occurring together) get heightened attention from media, government and community and can affect performance of immunization program. In commitment to improve vaccine safety, this paper summarizes a report of a cluster investigation for AEFIs (90 reports) following Japanese Encephalitis (JE) vaccine given in a mass campaign in one district (Morigoan of Assam), India in June, 2014.

Methods & Materials: In response to received reports of AEFI cluster over 10 day's period in June 2014, National AEFI surveillance team had investigated the reason for the events in the field by interviewing community and reviewing hospital records. Data of the individual cases was entered in anonymized line list and analyzed by using SPSS vs. 16.

Results: In Morigoan, 200574 doses of JE vaccine was administered in 1- 15 years age group during 15 days period in 2014 in mass campaign and among these 89 of the cases have reported symptoms of dizziness, tingling and numbness and abnormal movement of limbs. More than two- third of the affected individuals were females having median age of 9 years. Cases recovered without any residual sequel after receiving conservative treatment, reassurance and counselling in hospital.

Conclusion: There is a need of multi-pronged, effective information, education and communication intervention to handle unwanted rumors to ensure vaccine confidence during mass campaign by involving multiple stake holders.

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The seroprevalence of neutralizing antibody against Japanese encephalitis virus in health care workers

W.P. Vandepitte[1,*], S. Yoksan[2], M. Wannachart[1]

[1] *Queen Sirikit National Institute of Child Health, Bangkok, Thailand*
[2] *Institute of Molecular Biosciences, Nakhonpathom, Salaya, Thailand*

Background: Despite the introduction of inactivated Japanese encephalitis (JE) vaccine since 1990, JE remains an important cause of viral encephalitis in Thailand. Little is known about JE serostatus among Thais who were born before the 2001, the year that this vaccine has been included in the expanded immunization program. Therefore, the objective of this study was to determine the proportion of healthcare workers (HCWs) aged 21-60 years with adequate neutralizing antibody against JE virus.

Methods & Materials: We conducted a seroprevalence survey among HCWs during the routine annual check-up for HCWs at Queen Sirikit National Institute of Child Health (Children's Hospital, Bangkok, Thailand) during the period between July-October 2015. A purposive sampling was done to enroll a relatively equal number of 4 different age ranges i.e., 21-30, 31-40, 41-50, and 51-60 years per each group. JE serostatus was determined using 50% Plaque Reduction Neutralization Test (PRNT). Immunity to JE were quantitated and cross-tabulated against age, gender, past and present domicile, history of JE vaccination, types of vaccine received (if any).

Results: A total of 400 HCWs among a total of 1,320 who received annual check-up were enrolled. Only 1.5% of participants reported having immunized with JE vaccine. 80.5% demonstrated an adequate existing antibody against JE virus genotype 3 Beijing strain (at least 10 reciprocal PRNT titer). The proportion of protective antibody (and corresponding geometric mean titer) of the 4 age groups were 77.0% (112.48), 82.4% (211.47), 80.6% (84.59), and 82.0%(126.97) among those aged 21-30, 31-40, 41-50, and 51-60, respectively. Male gender was the only parameter that significantly associated with the lack of protective JE antibody with risk ratio and 95% confidence interval of 1.69 (1.1, 2.7).

Conclusion: Since JE virus is unlikely to be transmitted in hospital settings, the result from this group might be reflective of those in the general adult population in Bangkok. As a result, approximately